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# Is hyper-aggressiveness associated with physiological hypoarousal? A comparative study on mouse lines selected for high and low aggressiveness

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## ABSTRACT

Aggressiveness is often considered a life-long, persistent personality trait and is therefore expected to have a consistent neurobiological basis. Recent meta-analyses on physiological correlates of aggression and violence suggest that certain aggression-related psychopathologies are associated with low functioning of the hypothalamo-pituitary-adrenal (HPA) axis and autonomic nervous system (ANS). We tested this hypothesis in mice selected for high and low aggressiveness by measuring baseline plasma corticosterone levels and, via radiotelemetry, heart rate and core body temperature. The radiotelemetric recordings were made for 48 h under baseline undisturbed conditions and for 90 min after a handling stressor. Consistent with the hypoarousal hypothesis of violence, we found lower resting heart rates in two out of the three highly aggressive selection lines. In contrast, body temperature during the active phase, as another ANS-regulated physiological parameter, was higher in two out of three highly aggressive lines. The handling-induced tachycardiac and hyperthermic responses were similar across the six mouse lines except for the most docile and obese line, which showed a blunted reactivity. Besides significant differences between strains, no differences in plasma corticosterone levels were found between the high- and low-aggressive phenotypes. These results are discussed in relation to the different types of aggression (normal versus pathological) exhibited by the three highly aggressive lines. We conclude that while high trait-like aggressiveness is generally associated with a higher active phase core body temperature, only animals that express pathological forms of aggression are characterized by a low resting heart rate.

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## 1. Introduction

Aggressiveness is a behavioral trait used by individuals in competition with each other for vital resources such as food, territory, and mates, and to communicate social status [1,2]. When appropriately displayed, aggressive behavior serves an important biological function in securing these resources to reproduce successfully and transmit genes, and thus becomes evolutionarily conserved [3]. However, when expressed in an escalated manner, it is intense, frequent, injurious, and may lose its function in social competition and communication. In humans, this intense aggressiveness is referred to as violence, and is recognized as a pathological condition that requires treatment and prevention [4,5]. In order to develop successful intervention programs, it is necessary to understand the neurobiological causes of violence and their generality with respect to different forms of aggression and different environmental backgrounds [6].

It has been established that pathological aggressiveness in humans has a heritable component and that certain individuals have a pre-

disposition to engage in violent acts [7]. These individuals are aggressive and antisocial already in early childhood, their violent behavior progresses during adolescence, and it persists during adulthood. These so-called persisters differ from individuals that abstain from violence after adolescence [8].

The best-replicated biological correlate of life-persisting extreme aggressiveness so far is low resting heart rate [9,10]. Further characteristics include a low baseline activity of the hypothalamic-pituitary axis in delinquent adolescent humans and in violent rats [11,12], and deficits in prefrontal cortex functioning in antisocial psychopaths and violent rats [13–15]. Together, these data have led to the formulation of the “hypoarousal theory of pathological aggression”, which states that individuals with low physiological arousal engage more in violent acts throughout their lives as a form of stimulation-seeking behavior, without fear of punishment or aversive outcome [9,16].

While the literature concerning low resting heart rate and violence is quite consistent, autonomic reactivity to stressors seems to be considerably more variable. Hostile/aggressive Type A individuals show a positive correlation between trait aggression and emotionality measures [17,18]. Similarly, resident rats that readily attack male conspecifics have higher sympatho-adrenomedullary activation [19–21]. Similarly, in

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pigs, aggression levels and sympathetic activation during social confrontation are positively correlated [22].

Lines of mice selected for heightened aggressiveness, but originating from different strains, have been characterized in a comparative way in terms of their more or less violent aggressive phenotypes [23–27], and may therefore represent a useful model to investigate the physiological correlates of pathological aggression. The high-aggressive Short Attack Latency (SAL), Turku Aggressive (TA), and North Carolina 900 (NC900) male mice show similarly high frequencies and long durations of offensive aggression and short attack latencies to male docile intruders in their home cage. However, only SAL males show no discrimination based on the opponent's sex, fiercely attacking unfamiliar and familiar females, and very little discrimination in response to the opponent's inhibitory cues [26]. TA and NC900, although showing high levels of offensive aggression, do not attack females. In addition, while TA, like SAL mice, show a high motivation to maintain an offensive interaction, NC900 show a more fragmented pattern of social interactions with the intruder [26,27].

To date, information on the autonomic and neuroendocrine physiological phenotype of these lines is scarce. Regarding autonomic sympathoadrenal functioning, adrenaline content in the adrenals and in the brain stem of TA mice is higher than that of the corresponding low-aggressive Turku Non-Aggressive (TNA) mice [28]. When exposed to injection stress, core body temperature in SAL, TA, and NC900 male mice responds more than in the corresponding low-aggressive lines [24]. Regarding the HPA axis, baseline corticosterone levels are similar between SAL mice and their low-aggressive LAL (Long Attack Latency) counterparts, but SAL males show a substantially reduced reactivity to stress [29]. Studies on the immune system and aggression have revealed a lower sensitivity of the HPA axis to neonatal endotoxin exposure in NC900 compared to the low-aggressive NC100 mice, as well as lower serum corticosterone levels during handling [30,31].

The aim of the present research is to characterize the physiological phenotypes associated with these three different types of highly aggressive mice and to examine the generality of the hypoarousal hypothesis of violence. In accordance with the literature cited above, we expect low resting and low stress reactivity values for the physiological parameters under study in all three high-aggressiveness lines.

## 2. Materials and methods

### 2.1. Animals and experimental design

For this experiment, we used male mice of the aforementioned lines obtained through three different breeding programs for the selection of high-aggressiveness (SAL, TA, NC900) and low aggressiveness (LAL, TNA, NC100) [32–34]. The mice were bred in our own laboratory at the University of Groningen, The Netherlands, and kept after weaning (at 21 days of age) in unisexual familiar groups in Makrolon cages (type II). At 50 days of age, each male mouse was paired and caged with a female of the same line, to avoid male–male competition and social isolation. All the males were tested for attack latency using the standard procedure previously described [34]. Age-matched docile albino laboratory mice of the MAS-Gro strain were used as opponents.

For the corticosterone assay,  $n=5$  mice of each line were decapitated at the beginning of the dark phase under CO<sub>2</sub> anesthesia, and their trunk blood collected (for further details, see Corticosterone assay).

For the telemetry recordings,  $n=5$  or 6 male mice were implanted with a radio transmitter (for details, see Transmitter implantation and biotelemetry setup). Heart rate, core body temperature and activity were recorded around the clock to assess baseline physiology, whereas stress physiology was assessed from the heart rate, temperature and activity response to a brief handling challenge.

During the entire experiment, the animals were kept under standard laboratory conditions, at a temperature of  $22 \pm 2$  °C and on a 12:12 light–dark cycle (light on at 00.30), with food (AM11, ABdiets, Worden,

The Netherlands) and water available *ad libitum*. The experiments were approved by the Institutional Animal Care and Use Committee of the University of Groningen, the Netherlands, in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

### 2.2. Corticosterone assay

Trunk blood was collected in chilled tubes containing EDTA for determination of corticosterone levels. Blood samples were centrifuged at 2600 g for 10 min at 4 °C. Plasma samples were stored at  $-20$  °C until assayed. Plasma corticosterone was determined in duplicate using Immuchem™ Mouse Double-antibody Corticosterone <sup>125</sup>I RIA Kit, MP Biomedicals, LLC, Diagnostics division, Orangeburg, NY, US. The minimum detectable dose of corticosterone using this assay was 7.7 ng/ml, with an intra-assay variation coefficient of 4.4% and an inter-assay variation coefficient of 6.5%.

### 2.3. Transmitter implantation and biotelemetry setup

For the biotelemetry recordings, a TA10ETA-F20 mouse transmitter (DSI, St. Paul, MN, US) was implanted surgically in the intraperitoneal cavity of each male mouse ( $n=5$  or 6 for each line) after reaching at least 20 g of body weight. During the surgery, the mice were anaesthetized with a mixture of isoflurane (5% to induce and 3% to maintain) and NO<sub>2</sub>/O<sub>2</sub>, and placed on a Harvard heating pad to avoid hypothermia. The surgery was performed as previously described in [24]. Immediately after surgery, each mouse was placed in a clean cage under a heating lamp to avoid post-operative hypothermia. Cages were placed on a platform receiver connected via a matrix to a computer running Dataquest Labpro software for data collection. The mice were then allowed to recover undisturbed in their home cage for 4–6 days alone, and for the following week with their female partner. During this period, body weight and physiological parameters were monitored to ensure complete recovery and the presence of physiological circadian pattern.

### 2.4. Data collection and analysis

Plasma corticosterone levels were compared using a two-way ANOVA with type (two levels: aggressive and non-aggressive), strain (three levels: SAL/LAL, TA/TNA, NC900/NC100), and type\*strain interaction as between-subject effects. Post-hoc Tukey's pairwise comparisons were performed to decompose significant effects of factors with more than two levels.

Baseline recordings of heart rate, temperature and activity were obtained by sampling segments of 10 s every 5 min for a period of 48 h in which the animals were left undisturbed in their home cages. For heart rate and temperature, the 24-hr best-fitted curve was obtained using a linear harmonic regression fit that describes the data by adding harmonics to the principal wave function [35]. Averages of maximum, minimum, and amplitude were computed and compared within each pair of selected lines using *t* tests for independent samples.

Stress data were collected as response to handling, which consisted of lifting the experimental animal and placing it on a scale in order to measure body weight and immediately afterwards putting it back in its home cage. All handling on a given day took place in a 15-min time window between 10.00 and 14.00. Before and after handling, heart rate and temperature data were sampled every 5 min and logged automatically by a computer. The 60 min before the experimenter entered the room was considered a 'pre-stress' period, which was averaged in the analysis to give one datapoint (pre-handling). After handling, stress response was monitored for 90 min (post-handling), which was sufficient time to see a return to pre-stress values. Within each strain, the response curve of the aggressive line was compared with that of the low-aggressive line using a repeated-measures

ANOVA with time (19 levels) and type (aggressive and non-aggressive) as within- and between-subject factors, respectively. From the heart rate and temperature time-response curves, the area under the curve (AUC) was computed to obtain an overall response measure. For the overall response in activity, the sum of the 60-min baseline (pre-handling) activity was subtracted from the sum of the 90-min response (post-handling) activity. AUCs and total activity group averages were compared within each pair of selected lines using *t* tests for independent samples.

### 3. Results

#### 3.1. Attack latency test

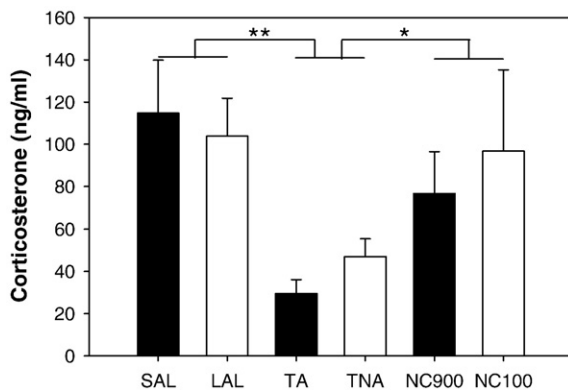
The behavioral test for aggressiveness, in which the animals had to face the challenge of a male intruder in their home cage, confirmed the highly aggressive phenotype of the SAL, TA, and NC900 lines and the low-aggressive phenotype of LAL, TNA, and NC100. All mice of the three high-aggressiveness lines quickly attacked the male intruders (attack latency in seconds, mean  $\pm$  S.E.M.: SAL =  $15.54 \pm 3.81$ ; TA =  $77.04 \pm 25.49$ ; NC900 =  $83.53 \pm 30.44$ ). As previously shown [24,26] among the low-aggressive lines, TNA mice showed a considerable amount of aggressiveness in terms of the number of attacking mice per line (LAL = 0/11, TNA = 6/11, NC100 = 1/11) and average attack latency (LAL =  $300 \pm 0$ , TNA =  $209.94 \pm 31.51$ , NC100 =  $295 \pm 5$ ). However, attacking TNA mice spent significantly less time in offensive behaviors than the highly aggressive lines (one-way ANOVA on all the attacking mice:  $F_{4,32} = 8.65$ ,  $p < 0.001$ ; mean<sub>TNA</sub> = 16.45 vs. mean<sub>SAL</sub> = 46.51,  $t_{13} = -4.5$ ,  $p = 0.001$ ; mean<sub>TNA</sub> vs. mean<sub>TA</sub> = 51.96,  $t_{13} = -4.59$ ,  $p = 0.001$ ; mean<sub>TNA</sub> vs. mean<sub>NC900</sub> = 44.22,  $t_{11} = -3.42$ ,  $p = 0.006$ ).

#### 3.2. Corticosterone data

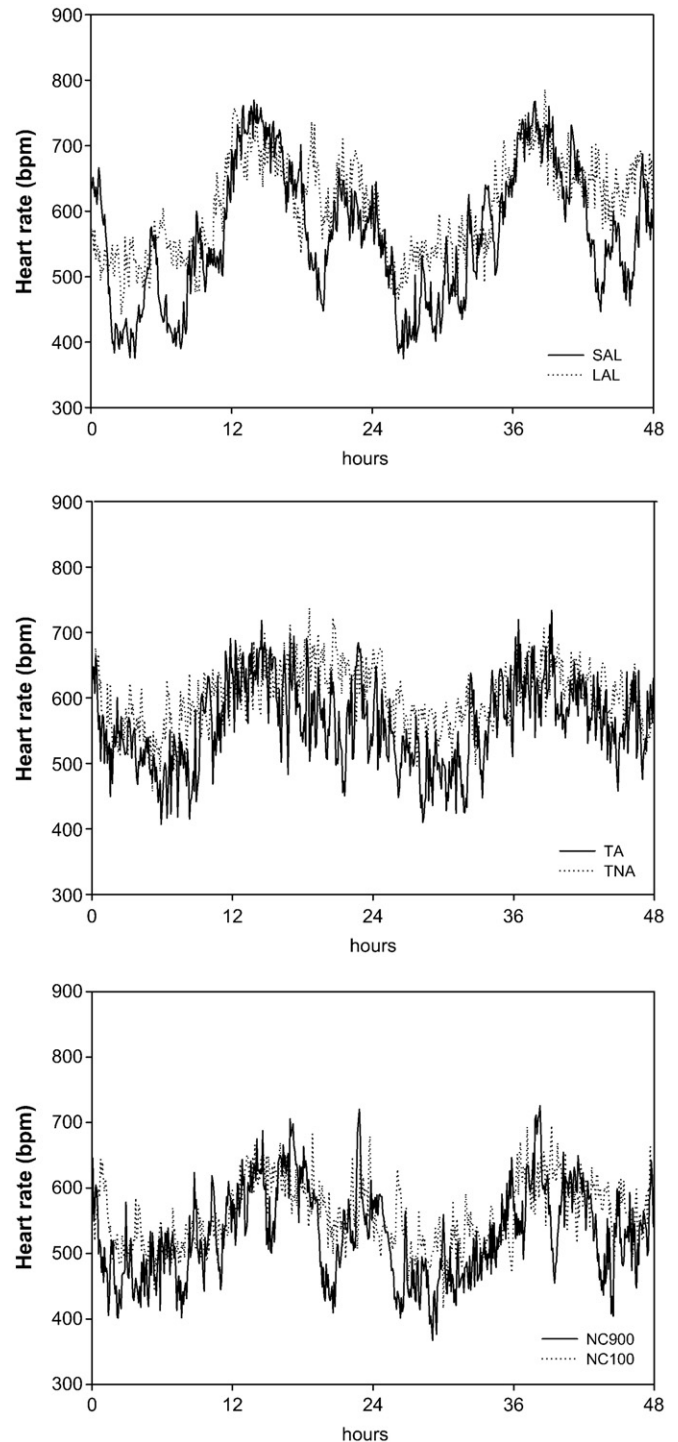
As shown in Fig. 1, plasma corticosterone levels were not associated with aggression. A two-way ANOVA on log-transformed values revealed a significant strain effect ( $F_{2,28} = 8.44$ ,  $p = 0.002$ ), which is due to the fact that TA/TNA mice had lower values than the other mice (compared with SAL/LAL, at  $p = 0.001$ ; compared with the NC lines, at  $p = 0.046$ ).

#### 3.3. Baseline physiology around the clock

As shown in Fig. 2 and summarized in Table 1, SAL mice had very low heart rates during their resting period. The minimum value was significantly lower in SAL than in LAL mice ( $t_{10} = -2.71$ ,  $p = 0.022$ ) and in TA compared to TNA mice ( $t_9 = -5.98$ ,  $p < 0.001$ ). The circadian wave in heart rate of the aggressive lines had a larger amplitude than that of



**Fig. 1.** Plasma corticosterone levels at the beginning of dark phase in SAL, LAL, TA, TNA, NC900 and NC100 mice ( $n = 6$  for each line). \* $p < 0.05$ , \*\* $p < 0.01$  with Tukey's pairwise comparisons.



**Fig. 2.** Circadian rhythm of average heart rate (bpm = beats per minute) in SAL, LAL, TA, TNA, NC900 and NC100 mice ( $n = 5$  or 6 for each line) measured every 5 min. Each panel shows two alternative mouse lines obtained through artificial selection for high (solid line) and low (dotted line) aggression. Standard errors were omitted for clarity.

low-aggressive lines, although significantly so in only TA compared to TNA (SAL/LAL:  $t_{10} = 2.2$ ,  $p = 0.052$ , TA/TNA:  $t_9 = 3.88$ ,  $p = 0.004$ , NC900/NC100:  $t_{10} = 1.94$ ,  $p = 0.084$ ).

As shown in Fig. 3 and in Table 2, active phase temperature values were significantly higher in the TA and NC900 lines compared to their low-aggressive counterparts (TA/TNA:  $t_{10} = 2.46$ ,  $p = 0.034$ , NC900/NC100:  $t_{10} = 2.39$ ,  $p = 0.038$ ), contributing to a difference in amplitude, with TA and NC900 having significantly bigger amplitudes than TNA and NC100, respectively (TA/TNA:  $t_{10} = 3.93$ ,  $p = 0.003$ , NC900/NC100:



**Table 1**  
Heart rate (bpm) during baseline conditions

Mouse line	Max	Min	Amplitude
<b>SAL</b>	<b>755.0±18.1</b>	<b>401.4±26.8*</b>	<b>353.6±23.0<sup>#</sup></b>
LAL	728.8±30.6	498.3±23.8	230.5±51.0
<b>TA</b>	<b>636.0±13.2</b>	<b>475.6±3.9**</b>	<b>160.4±10.4***</b>
TNA	649.2±9.0	550.4±10.8	98.8±10.9
<b>NC900</b>	<b>653.7±22.8</b>	<b>423.0±9.9</b>	<b>230.7±30.3<sup>#</sup></b>
NC100	632.8±41.3	498.1±46.9	134.7±39.0

*t*-test comparison with corresponding low-aggressive line.

\**p*<0.05, \*\**p*<0.01, \*\*\**p*<0.001, <sup>#</sup>0.05<*p*<0.1.

$t_{10}=4.15$ ,  $p=0.002$ ). Furthermore, in NC900, the resting phase temperature was significantly lower than in NC100 ( $t_{10}=-2.43$ ,  $p=0.035$ ).

The general activity levels (Fig. 4 and Table 3) were zero for all the mice at the minimum point of the cycle, which was during the light phase. The values for the dark phase were log-transformed in order to render the distribution normal and reduce the mean-to-variance relationship. No significant differences were revealed by *t* tests between the high-aggressive and low-aggressive lines (SAL vs. LAL:  $t_{10}=-1.82$ ,  $p=0.1$ ; TA vs. TNA:  $t_{10}=1.65$ ,  $p=0.1$ ; NC900 vs. NC100:  $t_9=-1.87$ ,  $p=0.09$ ). However, after removal of three outliers, the log-transformed activity levels were significantly higher in the LAL mice than in SAL ( $t_8=-2.56$ ,  $p=0.02$ ). In the other lines, no significant differences were found.

### 3.4. Physiological response to handling

Heart rate (Fig. 5) significantly increased in all lines due to the handling procedure (SAL/LAL:  $F_{18,180}=10.19$ ,  $p<0.001$ , TA/TNA:  $F_{18,180}=3.21$ ,  $p<0.001$ , NC900/NC100:  $F_{18,180}=5.63$ ,  $p<0.001$ ). A repeated-measures ANOVA did not show a differential change between aggressive and non-aggressive lines, although the overall response relative to baseline, measured as AUC, was lower in NC100 than in NC900 ( $t_{10}=2.88$ ,  $p=0.016$ ).

Temperature (Fig. 6) significantly increased in all lines due to the handling procedure (SAL/LAL:  $F_{18,180}=26.43$ ,  $p<0.001$ , TA/TNA:  $F_{18,180}=22.27$ ,  $p<0.001$ , NC900/NC100:  $F_{18,180}=26.14$ ,  $p<0.001$ ). All the aggressive lines had significantly higher values of temperature during the whole period (SAL/LAL:  $F_{1,10}=1.94$ ,  $p=0.012$ , TA/TNA:  $F_{1,10}=7.65$ ,  $p=0.012$ , NC900/NC100:  $F_{1,10}=16.23$ ,  $p=0.002$ ). However, the change was significantly different only between the NC900 and NC100 lines, as shown by the repeated-measures ANOVA (time\*type interaction effect:  $F_{18,180}=1.67$ ,  $p=0.048$ ) and analysis of the AUCs ( $t_{10}=3.98$ ,  $p=0.003$ ).

General locomotor activity (Fig. 7) increased after handling in all lines (SAL/LAL:  $F_{18,180}=5.37$ ,  $p<0.001$ , TA/TNA:  $F_{18,180}=6.85$ ,  $p<0.001$ , NC900/NC100:  $F_{18,180}=10.74$ ,  $p<0.001$ ). The increase in activity due to handling was lower in the SAL mice compared to LAL (time\*type interaction effect:  $F_{18,180}=1.78$ ,  $p=0.031$ ), and lower in the NC100 mice compared to NC900 (time\*type interaction effect:  $F_{18,162}=1.81$ ,  $p=0.028$ ). However, the total activity corrected for the baseline showed a significant difference only between NC900 and NC100 mice ( $t_9=3.98$ ,  $p=0.027$ ; analysis performed on log-transformed data to correct for non-normality of the distribution).

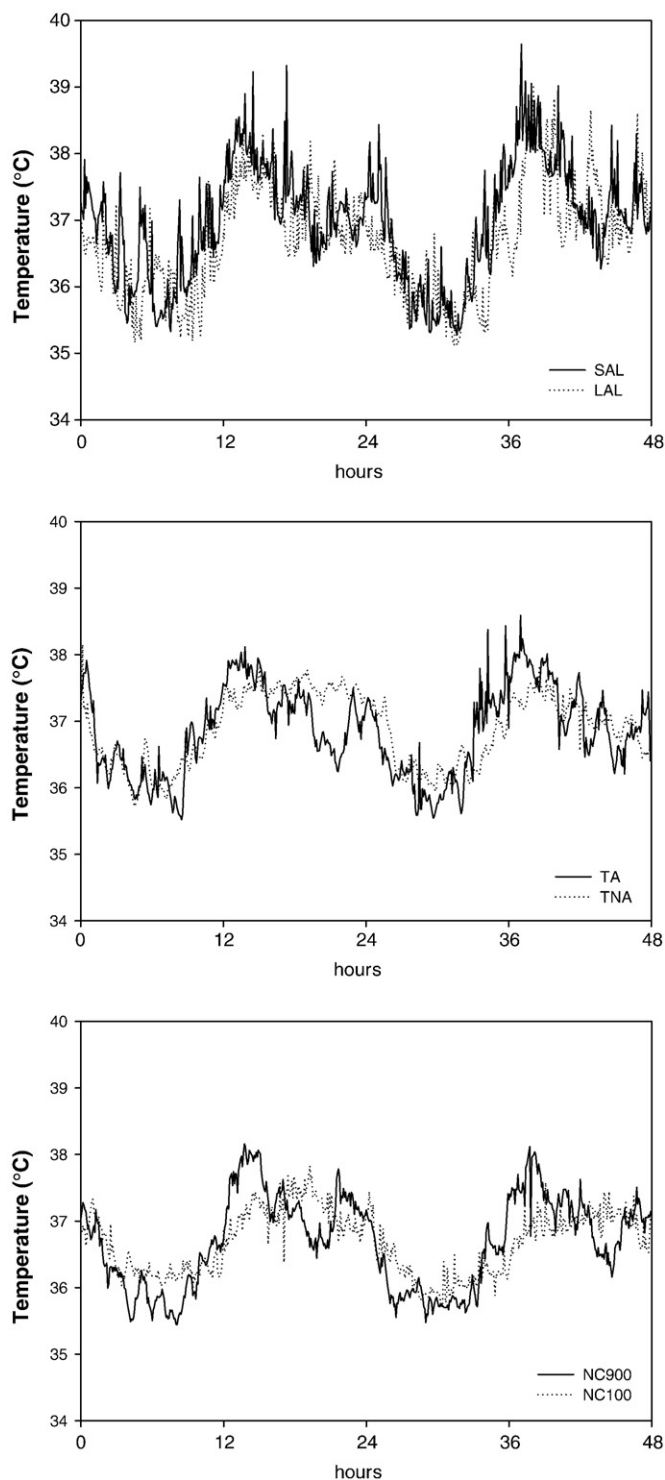
## 4. Discussion

Consistent with the hypoarousal theory of violence, low resting heart rates were observed in highly aggressive SAL and TA. These selection lines have previously been shown to be violent in terms of the sequential structure of agonistic interactions and insensitivity to inhibitory cues from the opponent [26,27]. High peak temperatures, as seen in TA and NC900 mice, seem to be associated with less violent forms of aggression. Aggression in these selection lines is still context-dependent, since it has been shown previously that they do

not attack familiar females in a novel cage or immobilized opponents [26,27].

### 4.1. Heart rate

As previously reported in other mouse strains, our lines showed clear circadian rhythms in heart rate, with the highest activity in the



**Fig. 3.** Circadian rhythm of average body temperature (°C) in SAL, LAL, TA, TNA, NC900 and NC100 mice ( $n=5$  or 6 for each line) measured every 5 min. Each panel shows two alternative mouse lines obtained through artificial selection for high (solid line) and low (dotted line). Standard errors were omitted for clarity.

**Table 2**  
Temperature (°C) during baseline conditions

Mouse line	Max	Min	Amplitude
<b>SAL</b>	<b>38.3±0.08</b>	<b>35.5±0.21</b>	<b>2.76±0.21</b>
LAL	38.1±0.17	35.5±0.38	2.54±0.44
<b>TA</b>	<b>38.0±0.07*</b>	<b>35.7±0.15</b>	<b>2.31±0.15**</b>
TNA	37.7±0.09	36.0±0.10	1.68±0.06
<b>NC900</b>	<b>37.9±0.17*</b>	<b>35.5±0.12*</b>	<b>2.43±0.18**</b>
NC100	37.4±0.13	36.0±0.15	1.44±0.16

*t*-test comparison with corresponding low-aggressive line.

\**p*<0.05, \*\**p*<0.01.

dark phase. The lower resting heart rate in the aggressive lines is in agreement with another mouse study, in which 5-HT1B knock-out mice displayed an aggressive phenotype and a low resting heart rate [36]. Both our study and Bouwnecht's reported resting heart rate values lower than 500 bpm, while in other laboratory strains that typically display low levels of aggression the values remain around 500 bpm on average [37,38]. Overall, we can conclude that low resting heart rate is associated with high levels of trait aggression in mice. The difficulty in extrapolating these data to humans is that the various studies performed on human populations are very heterogeneous in their operational definitions of aggression, as well as in the characteristics of the samples in terms of age, sex, socio-economic status, substance abuse, conviction, and so on. However, recent reviews support an association in humans between low resting heart rate and high injurious aggression [9,10,39], high antisocial/aggressive behavior [39], and antisocial psychopathy [9]. Recently, this association has been confirmed by Popma et al. [12] and Raine [40].

In contrast, an association between aggression and resting heart rate was not found in our NC900/NC100 lines. We can interpret this one of in two ways. One possibility is that the high levels of aggression exhibited by the NC900 males are representative of normal mouse aggression, since male mice, in contrast to other rodent species and humans, display high levels of physical aggression [41]. Alternatively, the high levels of aggression in the NC900 mice could represent a reactive form of aggression, according to the distinction between proactive/instrumental and reactive/emotional behavior emphasised by [42]. In support of the first interpretation, NC900 mice showed no offensive behavior toward females, and higher sensitivity to the opponent's cues than the other aggressive lines [26]. Moreover, their 5-HT<sub>1A</sub>/serotonin system was not associated with their high aggression levels, in contrast to the other two more violent aggressive lines [24].

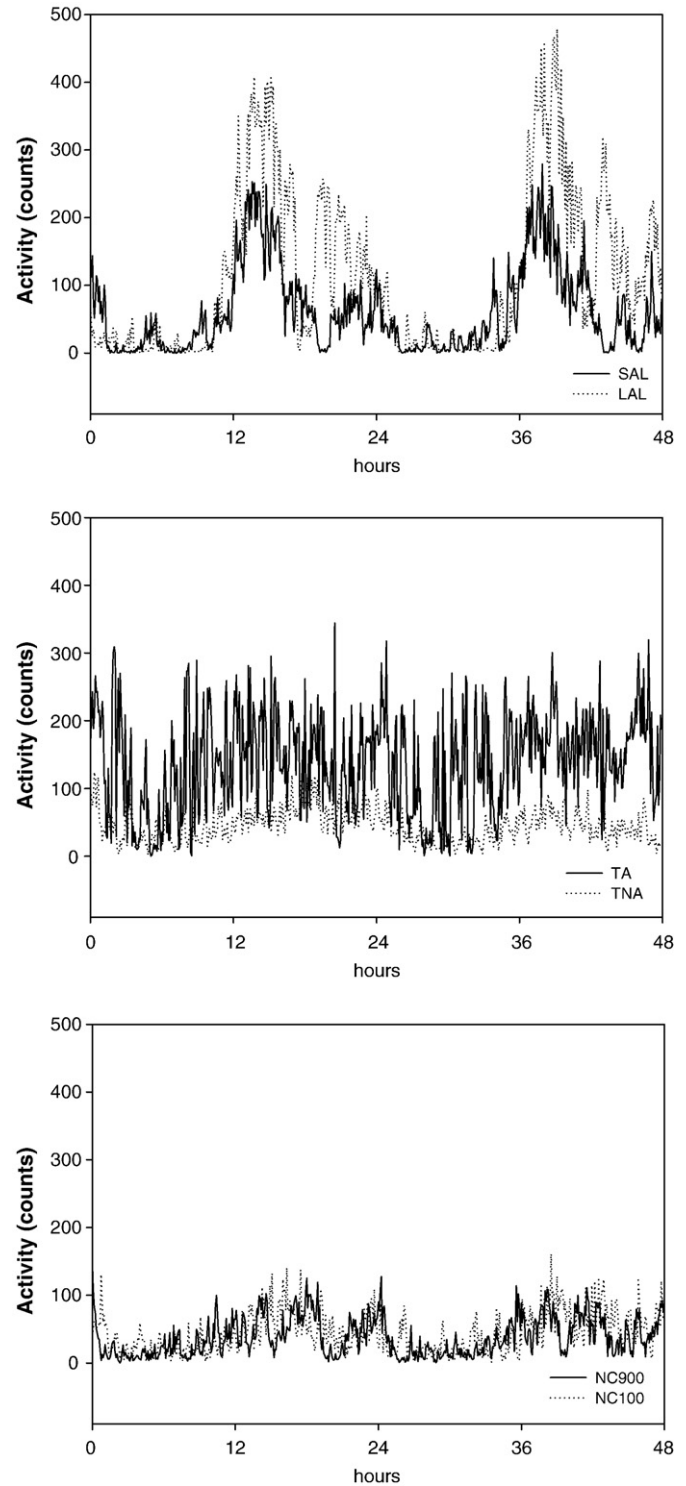
The most consistent finding regarding heart rate in this study is the larger circadian amplitude in the high-aggressive lines, compared to the low-aggressive lines. This may represent a superior physical fitness in the more aggressive lines [43]. Low resting heart rates in the most violent individuals strengthen this interpretation of superior physical fitness, since low resting rates are a typical characteristic of well-trained athletes in humans [44].

#### 4.2. Temperature and activity

As with heart rate, temperature showed daily variations with the highest peak occurring during dark phase. Both TA and NC900 mouse lines showed an association between high aggression and high peak temperature. Again, we can view the absence of this association in the SAL and LAL lines in one of two possible ways. First, one has to take into account the activity levels, since activity generates heat [45]. Higher average activity levels in the LAL line might raise their body temperature slightly during the active phase, bringing it up to values comparable to those of SAL mice. Higher activity levels in LAL were only partially seen in this study, but they have been shown in a previous study [46]. Alternatively, the high peak temperature/aggression pattern might be a characteristic of less violent forms of

aggression, mainly of the reactive type. Indeed, activity in the cage does not seem to have an effect on circadian rhythms in temperature, as previously shown in a rat telemetry experiment [47].

As with heart rate, the amplitude of daily temperature variation was greater in the TA and NC900 aggressive mice than their low-aggressive counterparts, though this pattern did not extend to the SAL/LAL lines. In general, we can conclude that aggressive mice display bigger amplitudes in their circadian rhythms.



**Fig. 4.** Circadian rhythm of average activity (counts) in SAL, LAL, TA, TNA, NC900 and NC100 mice (*n*=5 or 6 for each line) measured every 5 min. Each panel shows two alternative mouse lines obtained through artificial selection for high (solid line) and low (dotted line) aggression. Standard errors were omitted for clarity.

**Table 3**  
Activity (counts) during baseline conditions

Mouse line	Max	Min	Amplitude
<b>SAL</b>	<b>391±57.43</b>	<b>0</b>	<b>391±57.43</b>
LAL	652.3±133.5	0	652.3±133.5
<b>TA</b>	<b>553.5±226.8</b>	<b>0</b>	<b>553.5±226.8</b>
TNA	244.2±29.4	0	244.2±29.4
<b>NC900</b>	<b>344.8±42.8</b>	<b>0</b>	<b>344.8±42.8</b>
NC100	447.3±38.3	0	447.3±38.3

#### 4.3. Autonomic reactivity to stress

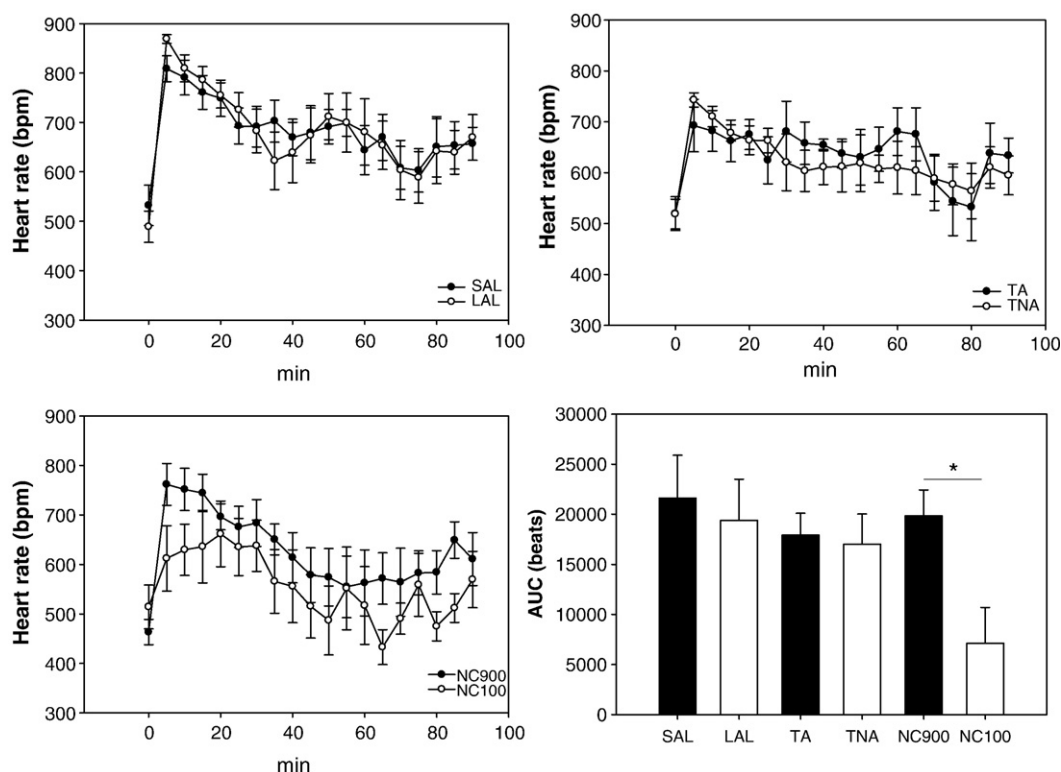
The handling experiment showed that only in the NC900/NC100 lines was aggression positively associated with higher heart rate and temperature reactivity to the stressor. As discussed earlier, selection for aggression may include a co-selection for higher reactivity to stress only in the case of discriminative aggression, and not in the most pathological selection conditions. In that case, the behavioral and physiological phenotype of these mice might then be more similar to the human reactive/emotional/hostile heightened aggressive type, rather than a mixture of instrumental/proactive and emotional/reactive types, which might be represented by the other two lines. Unfortunately this is difficult to determine, since the resident-intruder test for aggression is a typical challenge for reactive aggression, since the mice are threatened by the intrusion of an unfamiliar mouse in the cage. We have no information about the levels of instrumental aggression in these highly aggressive mouse lines. To our knowledge, a test for instrumental aggression in rodents has not yet been developed.

Alternatively, following a more careful interpretation, we should acknowledge that the stress-induced increase in temperature and heart rate was significantly lower in the NC100 non-aggressive line than in all the other lines, aggressive and non-aggressive (Temperature AUC: contrast<sub>30</sub>=3.22,  $p=0.003$ ; Heart rate AUC: contrast<sub>30</sub>=61.2,  $p=0.002$ ). This lower autonomic responsiveness might be more directly related to

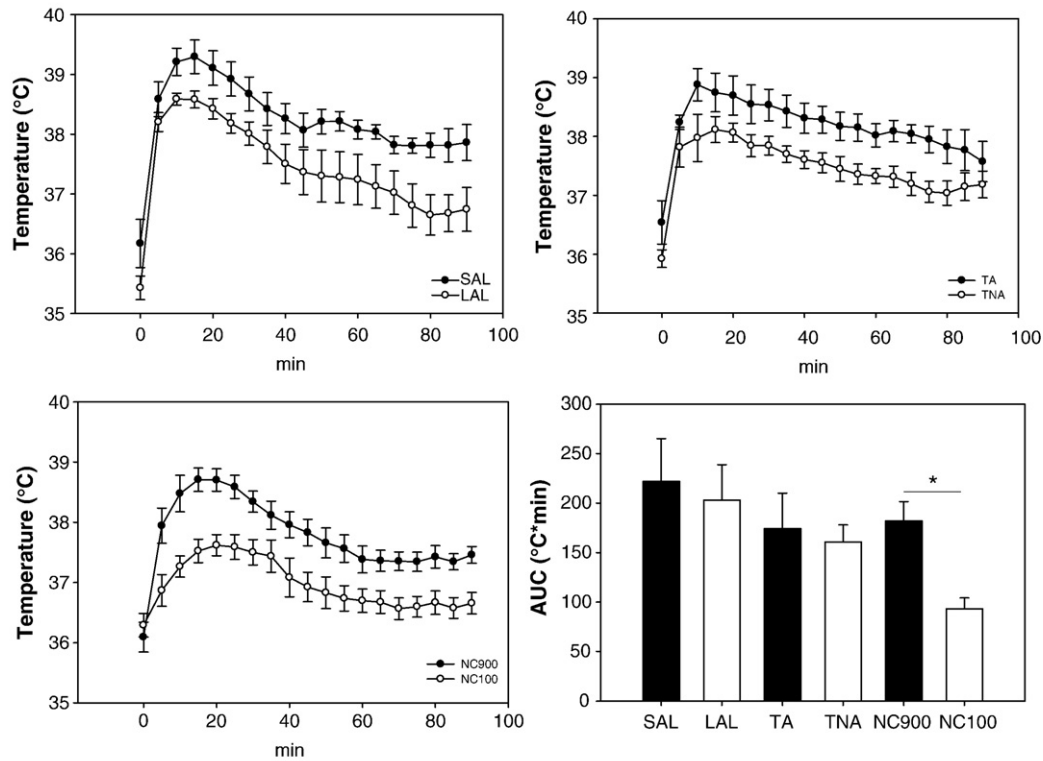
the fact that these mice may have developed some form of metabolic dysfunction related to obesity. In support of this hypothesis, the NC100 mice have a significantly higher body weight (means (g)±S.E.M: SAL=21.64±0.66, LAL=21.24±0.59, TA=34.1±0.6, TNA=30.06±0.52, NC900=37.78±1.33, NC100=48.82±2.5; NC100 significantly different from the other lines with  $t_{10}=-7.82$ ,  $p<0.001$ , planned contrasts corrected for inequality of variances), fairly low baseline activity levels (this paper), and higher leptin plasma concentration (means (ng/ml)±S.E.M: SAL=2.13±0.35, LAL=2.78±0.57, TA=3.02±0.44, TNA=2.34±0.54, NC900=3.58±0.45, NC100=5.8±0.72; NC100 significantly different from the other lines with  $t_5=-4.01$ ,  $p=0.012$ , planned contrasts corrected for inequality of variances). In line with these findings, it has been shown that diet-induced obese rats are hypo-responsive to stress [48]. This line of reasoning should be further explored in the obesity-prone NC100 mouse line.

The discrepancy between the three pairs of selected lines with regard to stress reactivity could also be explained in terms of specificity of the stressor. Handling is a routine procedure in the lab, a mild stressor that might be perceived with a similar degree of threat by mice with alternative behavioral phenotypes and that does not allow them to exhibit their alternative behavioral repertoires aimed to cope with the situation. It would be interesting to examine further the autonomic responsiveness in a situation where the mice can exhibit their higher or lower aggressiveness. Indeed, in rats, autonomic reactivity is positively associated with high levels of aggression [20], and the response is dependent on the stressor applied [49]. In humans, too, the higher autonomic reactivity to a stressor in type A individuals seems to depend on the stressor applied [18,50]. Hence, in line with previous research on mice [37], such a routine stressor as handling was stressful enough to elicit in mice a pronounced physiological response, consisting of hyperthermia, tachycardia and hyperactivity, and is therefore relevant for understanding stress-related autonomic activation.

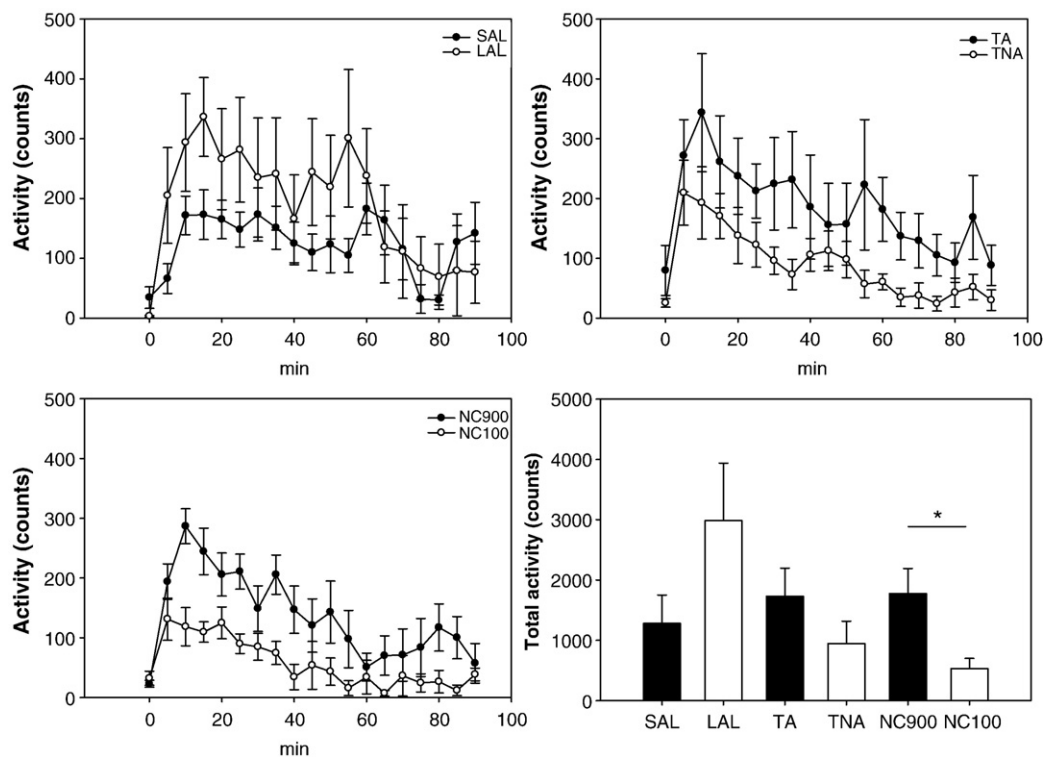
In conclusion, our study shows that different types of aggressive behavior are associated with different physiological traits. The selection



**Fig. 5.** Heart rate (bpm=beats per minute) in response to handling stress in SAL, LAL, TA, TNA, NC900, and NC100 mice ( $n=6$  for each line), depicted as group means ± standard error. Each panel shows two alternative mouse lines obtained through artificial selection for high (black) and low (white) aggression. The bottom-right panel shows the overall response calculated as AUC (=Area Under the Curve) for all the lines.



**Fig. 6.** Core body temperature (°C) in response to handling stress in SAL, LAL, TA, TNA, NC900, and NC100 mice ( $n=6$  for each line), depicted as group means  $\pm$  standard error. Each panel shows two alternative mouse lines obtained through artificial selection for high (black) and low (white) aggression. The bottom-right panel shows the overall response calculated as AUC (=Area Under the Curve) for all the lines.



**Fig. 7.** General activity (counts) in response to handling stress in SAL, LAL, TA, TNA, NC900, and NC100 mice ( $n=5$  or  $6$  for each line), depicted as group means  $\pm$  standard error. Each panel shows two alternative mouse lines obtained through artificial selection for high (black) and low (white) aggression. The bottom-right panel shows the overall response calculated as activity after handling minus activity before handling, for all the lines.



lines that exhibit aggression/violence in its most indiscriminate form are characterized by autonomic hypoarousal in the resting phase. Less pathological forms of aggression are related to hyper-arousal during the active period.

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